Indian J. Phys. **75B** (4), 333–336 (2001)

I J P B
— an international journal

Application of X-ray spectroscopic analysis to human blood samples

P K Hota*, V Vijayan[1], L P Singh[2]
Medical Physics Unit, AH Regional Cancer Centre, Cuttack-753 007, Orissa, India
[1]Institute of Physics, Sachivalaya Marg, Bhubaneswar-751 005, Orissa, India
[2]PG Department of Physics, Utkal University, Vanivihar, Bhubaneswar-751 004, Orissa, India
E-mail pradeephoto@hotmail com

Received 29 November 2000, accepted 24 March 2001

Abstract X-ray emission techniques like Particle Induced X-ray emission (PIXE) and Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry are applied to analyse the elements in biomedical samples at the Institute of Physics, Bhubaneswar in collaboration with the Acharya Harihara Regional Cancer Centre, Cuttack. After the development of the systems, initially experiments were carried out with the certified reference materials (CRMs) like IAEA animal blood standards to verify their applicability to biomedical samples Then human blood samples from different patients as well as healthy persons were analysed to compare the results applying both the techniques In this paper, both the X-ray emission techniques are discussed in detail with their advantages and limitations The results obtained by both the methods prove their efficiency for analysis of biomedical samples.

Keywords X-ray fluorescence, PIXE analysis, trace elements

PACS Nos. 87.64.Fb, 82.80.Ej

1. Introduction

Elemental analysis in biological systems is undertaken (a) to elucidate the fundamental biochemical links between chemical elements and living things, (b) to assess the state of health related to environmental exposure problems including nutrition, (c) for medical diagnosis of diseases and to monitor the progress of a therapeutic regimen and (d) to tackle problems in the area of occupational hygiene and preventive medical endeavors [1]. The emission of characteristic X-rays following atomic excitation has long been used for elemental analysis. The essential components of an X-ray spectrometer are an excitation source, a specimen chamber, a detection system capable of separating the X-rays by either wavelength, energy, or both, and the electronics necessary to process signals and accumulate spectra.

Analytical techniques based on X-ray Spectrometry (XRS) have been applied to the analysis of some elements in biomedical samples. These techniques are based on the detection and measurement of X-rays emitted from metal atoms when a vacant inner electron shell (primarily the *K*, *L* and *M* shells) captures outer shell electrons. This capture occurs when some or all of these inner shell electrons have been displaced by some form of irradiation or particle bombardment, *e.g.* high-energy photons (from an X-ray or radioisotopic source), electrons, protons, *etc.* Depending on the energy of the excitation source, an individual metal may emit relatively few X-rays of characteristic energy, which is then quantified. Electronic energy discrimination and multi-channel analysis permit simultaneous multimetal analysis by these techniques [2].

When X-ray photons are used as the excitation source, the technique is called X-ray fluorescence (XRF) analysis. In XRF, fluorescence spectra can essentially be measured in two ways such as Wavelength Dispersive XRF (WDXRF) and Energy Dispersive XRF (EDXRF). The most well known form of XRF uses X-ray tube excitation and has been widely used for environmental samples. When a dispersion grating is used to separate the secondary X-rays from the sample into a spectrum, it is known as WDXRF and when a solid state Si(Li) detector is used, it is known as EDXRF.

*Corresponding Author

In XRF analysis, the detection limits are tens to hundreds of micrograms per gram, and even with sample pretreatment, the detection capability is usually limited to a range between 0.01 to 10 μg/g. The detection limits are sufficiently low as to permit a survey type of analysis [1].

Similarly, when the excitation source is heavy charged particles, the technique is known as Particle Induced X-ray Emission (PIXE) analysis. The absolute detection limit of PIXE can be below the picogram level when special procedures are used. However, in real samples, depending upon the experimental conditions, absolute detection limits usually vary from 10-1000 mg. In life sciences, its applications are related to many trace elements in human and animal tissue samples. The major advantage of PIXE are its multi-elemental character (all elements from Na to U can in principle be measured), its high sensitivity (absolute detection limit is 10^{-12} g and relative detection limit is 0.1 μg/g), the smooth variation of the relative detection limit with atomic number of the analyte element, the ability to analyse tiny samples (1 mg or less), the speed of analysis (1–10 minutes bombardment time per specimen), the possibility for automation and the fact that it is often non-destructive [3].

Compared to X-rays, protons or other heavy charged particles have the advantage that electrostatic or electromagnetic lenses can focus them and they may be transported over large distances without loss of beam intensity. Hence compared to EDXRF, PIXE offers detection limits, which are often one order of magnitude better [4]. It allows one to analyse smaller sample masses and it is faster. XRF suffers from its inability to detect very light elements and unsatisfactory accuracy in the determination of phosphorus. Matrix effects are strong in some cases (*e.g.*, environmental samples) and in biological samples it is necessary to remove the inert elements like C, H, O and N. Moreover, preconcentration (*e.g.* ashing) becomes a prerequisite if overall improvement in sensitivity is to be achieved [1].

2. Material and methods

In this study, whole blood samples were collected from different patients suffering from different types of cancers, asthma, and diabetics as well as healthy persons to analyse elemental variations in their blood and study their possible role in different diseases.

2.1. Sample preparation :

About 5 ml of blood samples from each person under study were collected and lyophilised at the Regional Medical Research Centre (RMRC), Bhubaneswar. Then samples were converted into fine powder, homogenised and finally pressed into pellets. Conversion of the samples into a fine powder before pelletising is necessary to avoid particle size effects in the analysis. These effects are particularly important for the lighter elements. To obtain consistent pellets which remain stable during bombardment, an equal amount of binder such as cellulose (for EDXRF analysis) or graphite (for PIXE analysis) are added to the powdered sample prior to pelletisation. In PIXE analysis, a graphite binder has the advantage that the pellet is made conductive so that bombardment in vaçuum does not lead to charging of specimen (and thus to a high electron Bremsstrahlung background in the PIXE spectrum) [3].

For EDXRF analysis, the mass and diameter of each pellet were made 1 gm and 25 mm respectively, where as for PIXE analysis those were 300 mg and 13 mm respectively. Similarly, IAEA A-13 blood standards were also made into pellets to verify the accuracy of this method when applied to biomedical samples.

2.2. EDXRF analysis .

The EDXRF system at the Institute of Physics (IOP), Bhubaneswar used for the analysis of heavy elements incorporates a low power air-cooled X-ray tube (50 watt) as an excitation source with tri-axial geometry [5]. In this study, the X-ray tube was operated at 30 kV and 0.6 mA. The X-rays from the tube was exposed on a molybdenum secondary excitor and the *K*-characteristics X-rays of molybdenum were used to excite the characteristic X-rays of elements present in all the samples. The characteristic X-rays of elements present in each sample were collected using a Si(Li) detector and signals were processed by an amplifier and the spectra were recorded by using a PC-based multi-channel analyzer [5]. The spectra obtained were analyzed for all the samples. Each X-ray spectrum was corrected for the background. The photo-peak areas in each spectrum were evaluated using the computer program AXIL [6] supplied by International Atomic Energy Agency (IAEA) Matrix effects were taken into account in the spectrum deconvolution to obtain net intensities. The intensities of the characteristic X-rays of elements in the samples were proportional to the original concentrations in the samples, which were evaluated by least square fitting method using the AXIL program.

The elemental concentrations were determined using the equation,

$$m_j = N_{ij} / I_0 G \varepsilon \sigma_{ij} \beta_i, \qquad (1)$$

where m_j is the concentration (g/cm^2) of j-th element present in the sample, N_{ij} is the net counts per unit time for the i-th group of X-rays of j-th element, I_0G is the intensity of the exciting radiation incident on the sample visible to the detector, ε is the detector efficiency for the j-th element, σ_{ij} is the theoretical X-ray fluorescence cross section at 17.8 keV excitation energy and β_i is the self-absorption

correction factor that accounts for absorption of incident and emitted X-rays in the sample [7].

2 3. PIXE analysis :

Protons of 3 MeV, obtained from the 3 MV tandem pelletron accelerator facility at the IOP, Bhubaneswar, collimated to a beam of 3 mm diameter, were used to irradiate the targets. The irradiation was carried out under vacuum (10^{-6} Torr). The target holder can be moved in the vertical direction, which allows the analysis of different targets without changing the irradiation and measuring geometry. The targets were kept in the PIXE chamber at 45° to the beam direction. A Si(Li) detector with a FWHM of 170 eV at 5.9 keV (active area 30 mm^2, beryllium window thickness 12 μm, placed at 90° with respect to the beam direction) was used to detect characteristic X-rays emitted from the targets. X-rays leave the PIXE chamber through a 25 μm mylar window and traverse approximately 1 cm air gap before entering the detector. A 200 μm thick mylar absorber was kept in front of the detector to attenuate the Bremsstrahlung background and dominant low energy X-ray peaks. Spectra were recorded by using a Canberra series 88 multi-parameter analyzer [6].

Thick target PIXE analysis was performed using GUPIX-95 software, which provides non-linear least squares fitting of the spectrum, together with subsequent conversion of the X-ray peak intensities into elemental concentrations *via* a defined standardisation technique involving fundamental parameters and a user defined instrument constant. Full account was taken of matrix effects and secondary fluorescence contributions in both the spectrum fitting portion and the calculation of concentrations.

3. Results and discussion

Pellets made from the IAEA animal blood standard (A-13) were analysed by PIXE as well as EDXRF techniques for quantitative estimations of trace elements. The results are shown in Table 1 and the PIXE and EDXRF spectra for the IAEA blood standard are shown in Figures 1 and 2 respectively. It is observed from the table that the experimental values were in good agreement with the certified values.

Table 1. Results of X-ray analysis of the IAEA animal blood standard.

	Elemental concentrations (mg/kg) in IAEA Animal Blood (A-13) standard		
Elements detected	Certified value	Measured EDXRF-value	Measured PIXE-value
K	2500 ± 350	2596 ± 245	2429 ± 159
Ca	286 ± 53	291 ± 65	261 ± 27
Fe	2400 ± 150	2510 ± 182	2450 ± 206
Cu	4.3 ± 0.5	4.2 ± 0.3	4.2 ± 0.4
Zn	13.0 ± 1.0	13.3 ± 0.8	13.2 ± 1.3
Se	0.24 ± 0.08	0.21 ± 0.06	0.19 ± 0.14
Pb	0.18 ± 0.01	0.19 ± 0.09	0.20 ± 0.10

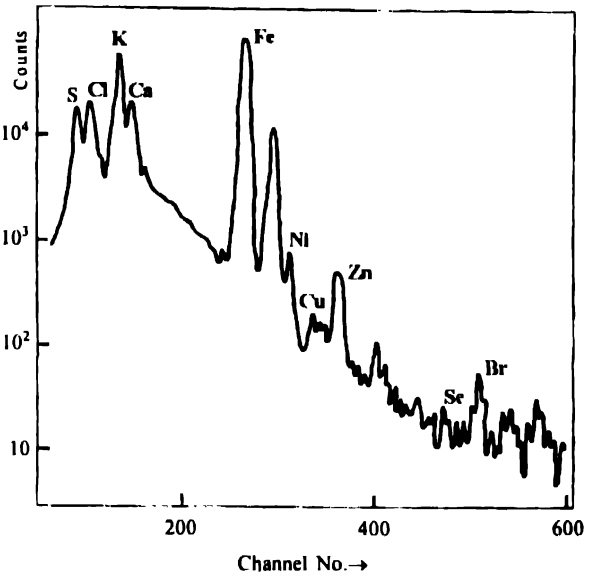

Figure 1. PIXE spectrum of IAEA (A-13) animal blood standard

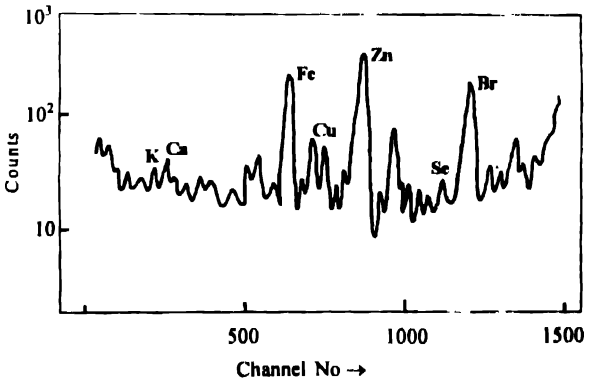

Figure 2. EDXRF spectrum of IAEA (A-13) animal blood standard.

Thus, it is clear that the experimental procedures adopted here were reliable for the study of these blood samples.

Table 2 shows the results of blood elements analysis of some patients as well as some healthy persons in both PIXE and EDXRF techniques. The results obtained for all cases applying both the methods were in order and almost matching. Thus, any of these methods can be applied for analysis of biomedical sampies and study of the essentiality and toxicity of any element in health and disease.

Human blood, blood plasma, serum, soft tissues, bones, human stones, hair, fingernails, skin and sweat used to be popular study objects for the X-ray spectroscopic analysis. In recent years, however, this type of application seems to be declining probably because of the fact that of the real trace elements only Fe, Cu, Zn and Se can be measured without pre-concentration [8]. Again, the need to have a particle

Table 2. Average elemental concentrations (± standard deviations) in human blood samples.

Elements detected	19 Healthy persons (Control)		13 Asthma patients	11 Diabetes patients	17 Leukemia patients	20 different cancer patients	
(in μg/ml)	PIXE	EDXRF	EDXRF	PIXE	PIXE	PIXE	EDXRF
K	1863 1	1938 7	2140 7	1702 1	1849.6	1798.8	1928.2
	(125 0)	(340 4)	(271.6)	(214 3)	(378.6)	(354 6)	(437.8)
Ca	270 8	271 7	319 9	264 0	289 5	380 1	290.8
	(135 4)	(95 7)	(126.3)	(111.2)	(80 6)	(58.4)	(65 6)
Fe	618 6	401 2	503 6	552.2	635 0	707.2	487.3
	(177 9)	(282 3)	(297.6)	(169 7)	(112 3)	(130.9)	(276.6)
Cu	0 94	0 77	0 76	0 90	1 02	1 12	0.71
	(0 17)	(0 47)	(0 41)	(0.47)	(0 24)	(0 24)	(0 44)
Zn	3 41	1 93	2 36	3 01	2 93	3 77	2.60
	(1 24)	(1 06)	(1 38)	(1 48)	(1 37)	(0.84)	(1 63)
Se	0.16	0 11	0 15	0 17	0 15	0 12	0 11
	(0 06)	(0 08)	(0 07)	(0 06)	(0.16)	(0.05)	(0.03)
Pb	0 08	0 07	0 10	0.11	0 12	0 14	0.09
	(0 04)	(0.02)	(0 04)	(0 05)	(0 04)	(0 04)	(0.02)

accelerator is a disadvantage in PIXE. Other limitations of the technique, which are also shared by EDXRF are that it suffers from spectral interferences, and that it does not allow the direct measurement of ultra-trace elements that are present at ng/g levels. Interesting elements which often remain undetected in biomedical samples are V, Cr, Co, Ni, As, Mo and Cd [3].

4. Conclusion

The results of PIXE and EDXRF analysis of IAEA standard biomedical reference materials prove that these techniques are efficient in generating data for trace elements in human blood samples. The feasibility to extend the use of X-ray emission analysis to biomedical samples has been established. A PIXE set up is very very expensive as it requires a particle accelerator. Even 100 mg of sample can be analysed by this technique. But, an EDXRF set up is not so expensive. It requires at least 500 mg of sample for analysis. Trace levels of essential elements, neutral elements and of certain toxic elements were detected in the midst of considerably larger (by several orders of magnitude) concentrations of major constituent elements by these techniques. This broad range of detection makes these X-ray emission techniques extremely useful in biology and medicine. Studies on trace element imbalances in different environment and different biomedical samples may lead to better diagnosis and treatment of human diseases.

Trace elements analysis in varieties of biomedical samples related to health and diseases can help and guide the clinicians to supplement or withdraw any element and mineral to the human body for cure of diseases and maintenance of better health. But, the researches on the role of elements and minerals in human health is lagging behind due to lack of adequate interaction between physicists, clinicians, biologists and availability of reliable analytical facilities like X-ray emission analysis.

References

[1] G V Iyengar *Elemental Analysis of Biological Systems* (Florida CRC Press) **Vol 1**, (1989)

[2] L W Chang *Toxicology of Metals* (Florida . CRC) p 76 (1996)

[3] Willy Maenhaut *Scanning Microscopy* **4** p 43 (1990)

[4] W Maenhaut and Klas G Malmqvist *Hand Book of X-ray Spectrometry* (eds.) Rene Van Grieken and Andrzej a Markowicz (New York : Marcel Dekker) **Ch. 11** p 517 (1992)

[5] P K Hota, V Vijayan and L P Singh *The Indian J. Nutr Diet* **37** p 214 (2000)

[6] V Vijayan, S N Behera, V S Ramamurthy, Sanjiv Puri, J S Shahi and N Singh *X-ray Spectrometry* **26** p 65 (1997)

[7] S Puri, D Metha, B Chand, N Singh, P N Trehan *Nucl. Instrum Meth* **B73** 319 (1993)

[8] Willy Maenhaut *Nucl. Instrum Meth.* **B35** 388 (1988)